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T-cell Differentiation: MHC Class I's Sweet Tooth Lost on Maturity

Dispatch

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The addition of sialic acid to O-linked glycans of the T-cell co-receptor CD8 is regulated during thymocyte differentiation. Two recent papers have shown that this glycosylation changes the avidity of the interaction between CD8 and MHC class I proteins, potentially altering signalling thresholds in thymocyte differentiation.

During T-cell development in the thymus, expression of the co-receptors CD4 and CD8 precedes that of the T-cell antigen receptor (TCR). CD4 and CD8 are expressed together at the immature stage (double positive, DP cells) after rearrangement and expression of the TCRβ chain gene on the cell surface as part of the pre-TCR, but before rearrangement and expression of the TCRα chain gene. During the DP stage, thymocytes rearrange TCRα genes until the TCRαβ protein combination is capable of recognising a ligand — a short peptide bound to a major histocompatibility (MHC) molecule — that is sufficient to transduce a signal for positive (or negative) selection. With signalling for positive selection, the cells differentiate to $CD4^+CD8^-$ or $CD4^-CD8^+$ single positive (SP) thymocytes, whereas negative selection results in deletion of thymocytes.

CD8 and CD4 are not simply markers of differentiation, but function as co-receptors for the TCR. Each interacts with MHC at a site distinct from that recognised by the TCR, with CD8 binding to MHC class I molecules and CD4 binding to MHC class II molecules. It is generally considered that these co-receptors interact with the same peptide–MHC molecule as is recognised by the TCR. However, they can also act as adhesion molecules, binding to MHC irrespective of the peptide bound to the MHC molecule [1]. Such 'non-cognate' binding has often been dismissed as an artefact of overexpression, but new data from the labs of Ellis Reinherz [2] and Steve Jameson [3] now show that the strength of non-cognate binding between CD8 and MHC class I molecules is developmentally regulated, being much stronger in immature DP thymocytes than in mature $CD4^-CD8^+$ SP thymocytes or mature peripheral $CD8^+$ T cells (Figure 1).

The glycosylation of CD8 is regulated during T-cell differentiation [2,4] and T-cell activation [5]. This is particularly evident with CD8β, where O-linked glycans become sialylated on activation [5] or on maturation from DP to SP subsets [2]. The lectin peanut agglutinin (PNA) binding is a venerable surrogate marker for thymocyte maturation [6]. PNA binds 'core 1' O-glycans with a terminal galactose moiety and binding is lost with addition of a sialic acid residue to the core 1 branch, or by addition of sugar residues to form 'core 2' branches on the glycan (Figure 2). PNA staining of thymocytes is primarily on DP cells, with PNA reactivity being lost upon maturation to SP cells. The main PNA-binding proteins on thymocytes are CD8, CD43 and CD45 [4]. Notably, the sialyltransferase ST3Gal-1, which adds sialic acid to the core 1 O-glycan Galβ1-3GalNAc-Ser/Thr is induced in medullary thymocytes, which represent the SP population ([7,8] and references in [9]).

MHC class I tetramers, made by crosslinking biotinylated peptide–MHC class I monomers with avidin have been used extensively to stain antigen-specific $CD8^+$ T cells. It is accepted that these tetramers bind only to T cells with a TCR that recognises the specific peptide−MHC ligand ('cognate binding') and, unlike MHC class II tetramers, where binding seems to be independent of CD4, CD8 aids in tetramer binding [10]. Therefore, it was a surprising result when Bosselut and colleagues [11] showed TCR-independent binding of MHC class I tetramers to CD8αβ on thymocytes. Comparison of tetramer binding on thymocyte subsets shows clearly that there is no binding to $CD4^+$ SP or $CD4^-CD8^-$ (double-negative, DN) cells. The DP cells show strong tetramer staining which is reduced in the subsets of this population where CD8 expression is decreased. Tetramer binding is strongly reduced in the CD8 SP cells [3]. Tetramer binding on the DP cells is independent of the MHC haplotype and of TCR: $TCR\alpha^{-/-}$ thymocytes are stained, even though they cannot express an αβ TCR [2,3]. Binding requires CD8β expression as part of the CD8αβ heterodimer, because there is no staining of either $CD8\alpha^{-/-}$ (no CD8 expressed on the cell surface) or $CD8\beta^{-/-}$ (CD8αα expressed on the cell surface) thymocytes [2]. Binding is blocked by both anti-CD8α or anti-CD8β antibodies [2,3].

Neuraminidase treatment of SP thymocytes to remove sialic acid greatly increases their binding to MHC class I tetramer, as well as slightly increasing the binding to DP cells [2,3]. In concert with this, PNA-binding sites are also revealed on the SP cells [2–4]. Mice deficient for ST3Gal-1 enzyme have little overt defect in thymocyte development, but have a loss of naïve mature $CD8^+$ peripheral T cells [9]. There is a deficiency in the number of mature, positively selected, $CD8^+$ SP thymocytes and the $CD8^+$ SP cells show increased binding of MHC class I tetramer, although not to the same extent as with neuraminidase treatment [2]. Thus it is clear that sialylation is responsible for the loss of the non-cognate CD8–MHC class I interaction that occurs after positive selection. More evidence that the change in the avidity of the

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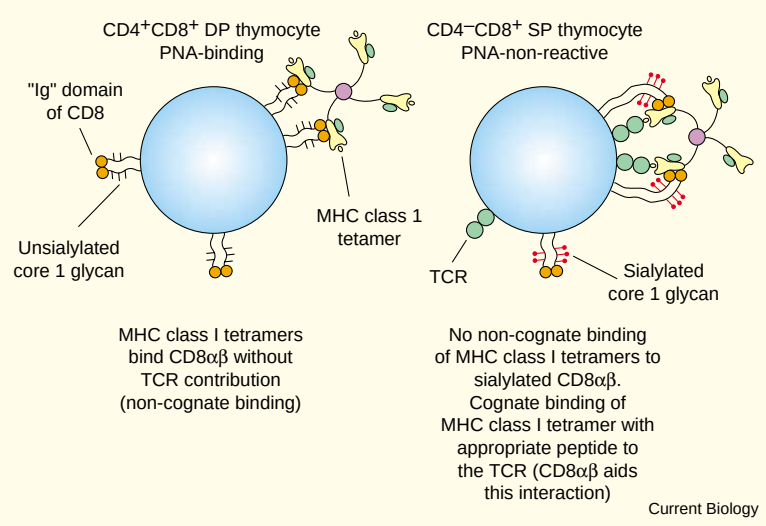

Figure 1. Non-cognate MHC class I binding to CD8αβ is regulated during positive selection.
The DP thymocyte (left) binds MHC class I tetramers irrespective of the peptide bound to the MHC class I molecule. The SP thymocyte (right) can only bind MHC class I tetramers if the peptide in the MHC groove is specifically recognised by the TCR of that cell. (Tetramer is shown binding through two MHC class I units for clarity.) This change in the binding properties of MHC class I molecules to CD8 is mediated by sialylation of CD8αβ, in particular of the CD8β chain. The relevant sialyltransferase, ST3Gal-1, is induced during DP to SP differentiation. Similarly, DP cells bind PNA due to lack of sialylation of membrane glycoproteins including CD8. PNA-binding is lost in the SP cells because of sialylation.

CD8–MHC class I interaction is regulated by positive selection comes from experiments comparing tetramer-staining of TCRα$^{-/-}$ thymocytes, OT1 TCR transgenic (OT1) thymocytes and thymocytes from OT1 mice deficient in the peptide loading protein TAP. Positive selection of DP thymocytes in OT1 mice is highly efficient, but is blocked in the OT1 TAP$^{-/-}$ thymus because of the lack of peptide loading onto MHC class I molecules, and as mentioned, the TCRα$^{-/-}$ thymocytes are unable to undergo positive selection because they have no TCR. Tetramer staining of both TCRα$^{-/-}$ and OT1 TAP$^{-/-}$ DP thymocytes is strong, but is reduced in the OT1 DP cells, indicating that active positive selection results in decreased tetramer reactivity [3]. The sialylation may also have an effect on repertoire selection because a small but statistically significant difference is seen in the TCRβ chain repertoire when comparing ST3Gal$^{-/-}$ to control animals [2].

Thus the interaction between CD8αβ and MHC class I molecules is turned off during the developmental programme by sialylation of core 1 O-linked glycans of CD8. The extracellular parts of CD8α and CD8β consist of an immunoglobulin-like recognition domain and a ‘stalk’ region where the O-linked glycans are located. It is thought that these glycans hold the structure in an extended conformation in order that the stalk can reach from the T-cell membrane to the membrane-proximal part of the MHC class I molecule on the antigen-presenting cell [5,12]. The role of CD8 sialylation in changing the avidity for MHC class I molecules is unknown. Moody and co-workers [2] have suggested that the sialylation forces CD8α and CD8β apart, thus reducing the ability to bind MHC class I, since the CD8αα–MHC class I interaction involves residues from both members of the αα dimer [13,14].

Another possibility relates to the formation of ordered arrays of molecules on the cell surface by glycan-binding, crosslinking proteins such as galectins, which could alter the availability of CD8 for binding the MHC class I tetramers. For example, galectin-3 binds to particular N-linked glycan structures and reduces T-cell activation by restricting TCR clustering [15]. Similarly, galectin-1, which recognises CD4 and other glycoproteins through core 2 O-glycans, has immunomodulatory effects similar to antagonist ligands [16]. Perhaps a similar interaction involving CD8 O-linked glycans could be altered by sialylation and therefore affect the CD8–MHC class I interaction.

The non-cognate CD8–MHC class I interaction presumably has a role in thymocyte development. As demonstrated by Daniels and co-workers [3], the interaction is sufficient for T-cell binding to an MHC class I surface. Earlier work showed that TCR activation of CD8$^+$ cells increases CD8–MHC class I non-cognate binding, and that the CD8–MHC class I interaction increases activity of the CD8-associated kinase Lck [17,18]. Activation of peripheral T cells results in reduction of sialylation [5,9], although no change in the non-cognate CD8–MHC class I interaction has been described. Immature thymocytes are more sensitive to stimuli than are mature cells [19,20], suggesting that the role of the non-cognate CD8–MHC class I interaction could be to pre-sensitise the cells so they can respond to a weak stimulus. Furthermore, the non-cognate interaction could pre-concentrate MHC class I molecules in the immunological synapse for testing by the TCR, thus compensating for the low level of TCR on pre-positive selection DP cells [2]. It remains to be seen whether analogous changes in non-cognate CD4–MHC class II interactions occur during thymocyte development, but this should be an area of strong interest in the near future.

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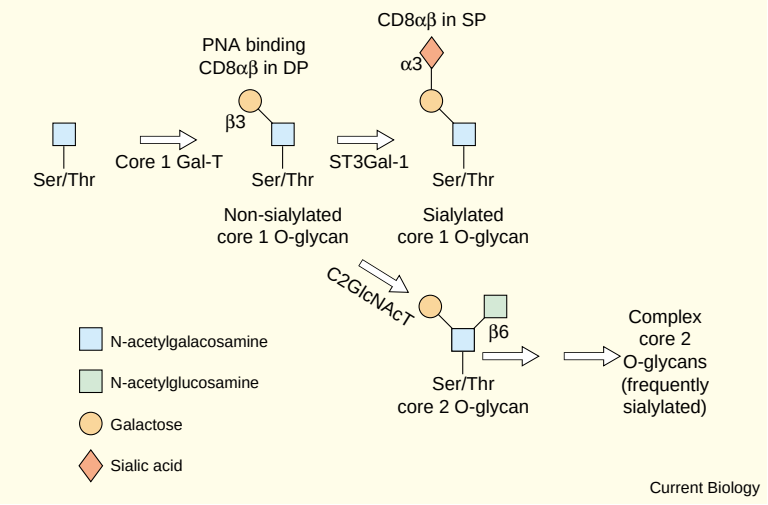
Figure 2. O-linked glycan synthesis involved in CD8αβ interaction with PNA and the non-cognate interaction with MHC class I molecules.

The unsialylated core 1 O-glycan is produced by core 1 galactosyltransferase, resulting in the structure recognised by PNA and required for non-cognate CD8αβ interaction with MHC class I molecules. The sialyltransferase ST3Gal-1 can then produce the sialylated core 1 O-glycan, which does not bind PNA and abrogates the non-cognate interaction between CD8αβ and MHC class I. The core 2 N-acetylglucosaminyltransferase (C2GlcNAc-T) has access to the structure after ST3Gal-1.



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